The value of cytogenetics for the taxonomy and evolution of Leaf Beetles (Coleoptera, Chrysomelidae)

E. Petitpierre

Petitpierre, E., 1997. The value of cytogenetics for the taxonomy and evolution of Leaf Beetles (Coleoptera, Chrysomelidae). *Misc. Zool.*, 20.1: 9-18.

The value of cytogenetics for the taxonomy and evolution of Leaf Beetles (Coleoptera, Chrysomelidae).– The advantages and pitfalls of cytogenetics for the taxonomy and evolution of Leaf Beetles are discussed. Karyology may provide clues for distinguishing cryptic sibling species as demonstrated in *Chrysolina aurichalcea* and *Cassida viridis*. The phylogenetic value of karyotypes can only be substantiated on the grounds of three general rules: extensive sampling to determine the most widespread karyotype or meioformula, the criterion of parsimony, and parallel evolution of other characters. A modal karyotype cannot be equalized *a priori* to the most primitive karyotype. Hence, for simple effects of sampling, in a few Leaf Beetle subfamilies only the ancestral karyotype can be reasonably assessed. The Chrysomelinae subfamily is studied in significantly greater detail, and the probable interrelationships of their higher taxa based on the chromosomal findings and their correlation with other characters of phylogenetic interest is discussed. The presumed effects of deme size and specialized phytophagy on the karyological evolution of Chrysomelinae genera are also dealt with.

Key words: Cytogenetics, Leaf Beetles, Chrysomelinae, Taxonomy, Evolution.

(Rebut: 10 I 97; Acceptació definitiva: 11 III 97)

Eduard Petitpierre, Lab. de Genètica, Dept. de Biologia Ambiental, Univ. de les Illes Balears-UIB, 07071 Palma de Mallorca, Espanya (Spain).

This work was supported by project DGICYT PB93-0419, Ministry of Education and Culture, Spain.

ISSN: 0211-6529

Introduction

The Chrysomelidae or Leaf Beetles are one of the largest families of Coleoptera with some 35,000 described species, and probably a great number as yet undescribed (Lawrence, 1982; Jolivet & Cox, 1996). This is the most important taxon of the superfamily Chrysomeloidea, where Cerambycidae and Bruchidae are also included among a total of five families (Crowson, 1981).

The recent publication of three books dealing with the biology of Chrysomelidae, in the broadest sense (Jolivet et al., 1988, 1994; Jolivet & Cox, 1996), and specially several of its chapters dedicated to the phylogeny and evolution of the group, has given rise to considerable interest in these subjects from a multidisciplinary view. Two authors specialized in Leaf Beetles recently questioned the usefulness of cytogenetics as a suitable tool for phylogenetic and evolutionary studies. Crowson (1994) stated that 'chromosomal patterns do not usually provide reliable markers for higher taxa ,... marked karyotypic differences may be found between species which in other respects seem closely allied, and very similar karyotypes can persist in extensively varied groups', to conclude 'the main systematic (and biological value) of karyotypic studies is likely to be around the level of species, as demonstrated e.g. by Virkki's studies in Alticinae'.

In addition, Reid (1995) did not use karyology among the 71 characters used in his long and detailed cladistic analysis of Leaf Beetles, because 'karyology tends to consider *common* to *primitive*, but this is unacceptable for several reasons, including sample bias and failure to distinguish between an ancestral state from one shared by a recent radiation of species', adding finally that 'at present levels of study, kariology is useless for chrysomelid phylogeny except at trivial levels, as already noted by Crowson (1981)'. Nevertheless the topic is worthy of more detailed study and discussion.

The value of karyotype for the taxonomy of Leaf Beetles

One characteristic of the phenotype of a species is its karyotype, defined by chromosome number, size and morphology of each individual chromosome, and when present, such as in most animals, the type of sex-chromosome system. The karyotype of a species is usually constant and can therefore be considered as a definite element, like any morphological character, for taxonomic purposes.

A simple manner to determine the rough karyotype of a beetle species is by the meioformula or karyotypic formula, the number of autosomal bivalent plus the type of sex-chromosome system, usually obtained from males, at meiotic metaphase I (Smith & Virkki, 1978). Thus, a meioformula of $9+Xy_p$, means nine autosomal bivalent and a sex-chromosome bivalent made up of a large X and a small y, noted by a capital letter and a small letter respectively, held together by a 'parachute' (p) type of association, because it resembles this configuration. Equality of meioformulas between two species does not mean they have identical karyotypes. If this were true the huge number of beetles with the modal $9+Xy_p$ meioformula would be indistinct in their karyotypes, which is clearly nonsense. In fact, almost all species can be differentiated in their karyotypes provided they are surveyed through a high resolution analysis, namely by chromosome banding, *in situ* hybridization, and a deep study of meiosis. The only known exceptions to this rule in animals (just over 1%), are the homosequential species of *Drosophila*, whose polytenic chromosomes enabled a high resolution analysis. They have identical patterns of bands and are therefore impossible to distinguish (White, 1978).

Specifically regarding Leaf Beetles, closely related species can differ or not in their meioformulas, but when they are coincident even a conventional morphometric analysis of mitotic metaphase chromosomes, which is not an extremely powerful technique, can demonstrate differences, as shown in two Indian species of *Cassida* (Yadav & Pillai, 1975), the allied species of *Chrysolina* feeding on Labiatae plants (Petitpierre, 1983), and 13 North American *Leptinotarsa* (Hsiao & Hsiao, 1983).

Both Crowson (1994) and Reid (1995) agree in the acceptance of the karyotype

as a valuable character to species taxonomy. Nevertheless, and contrary to the latter author, the species level is not at all trivial for taxonomy and phylogeny, either in basic or in applied research, and several examples could be quoted to support this view.

Two recent findings illustrate the value of karyotype for taxonomy and phylogenetic interrelationships. The first is represented by the complex of taxa known as *Chrysolina aurichalcea* from Japan and Korea, whose existence was proved by karyotypic analysis showing distinct chromosomal races, with n=32, 2n=42 and 2n=46 diploid numbers, that should be rightly considered as sibling species (PETITPIERRE, 1981; FUJIYAMA, 1989). The spermatids of siblings with n=16 and n=23 chromosomes had no significant differences in their DNA content, so, the chromosomal shifts involved in the origin of the latter from the former, according to the common trend found for the genus, have not changed this parameter (PETITPIERRE et al., 1991). A second example of siblings was found in the tortoise beetle *Cassida viridis*, the specimens from Switzerland and from Catalonia (NE Spain), dsiplayed 2n=24, while those from two geographical sources in Andalucia (S Spain) had 2n=30 chromosomes (PETITPIERRE et al., 1988, in prep.). Whereas the adults of *Chrysolina aurichalcea* siblings show slight differences in male genitalia and ecological niches (FUJIYAMA, 1989; FUJIYAMA et al., 1991; FUJIYAMA & TAKANASHI, 1994), those of *Cassida viridis* siblings are identical but their larvae have not as yet been studied and might provide some clues for distinction.

Greater efforts should be directed to the karyological characterization of Leaf Beetle species by using conventional techniques of staining to extend the sample analyses, and also high resolution techniques, especially in the taxa where conservative chromosome numbers and meioformulas are prevalent. Nevertheless, the karyotype is only a part of the genetic knowledge needed for an accurate definition of a species, along with the classic exophenotypic characters of morphology, life history, ecology and biogeography.

The karyotype as a phylogenetic character

Phylogeny implies recognition of polarity and the modern and extensive cladistic analyses in Leaf Beetles assume ancestral (plesiomorphous) and derived (apomorphous) states in many characters to build up the most parsimonious trees, phenograms or cladograms (LEE, 1993; REID, 1995). Likewise, cytogenetic data may be used in phylogenetic studies if polarity of change is reasonably inferred. Before discussing how to answer the question of polarity in evolutionary cytogenetics, several important premises should be clearly settled. First of all, there is not *a priori* reason for assuming that extant species with primitive morphology, behaviour and life history should also have the most primitive karyotypes. This may occur or not, because evolution proceeds in a *mosaic* way with some characters being derived and others conserved with respect to the ancestral state. A correspondence between karyotype and evolutionary ancestry could be true if the cytogenetic characters determine, at least to some degree, the states of the non-cytogenetic characters, and/or if the rates of karyotypic change and those of other characters are correlated across evolving lineages. Consequently, the cytogenetic characters cannot be taken alone in phylogenetic analyses, especially in taxonomic categories higher than genus. They can serve to test phylogenetic hypotheses principally based on exophenotypic characters, that is morphologic and behavioural traits, and this is their main evolutionary usefulness.

But how can we determine the direction of karyotypic change between two states A and B: A —> B or B —> A? A common practice, followed by several evolutionary cytogeneticists, has been to equalize the modal karyotype to the ancestral state. This presumed equality is obviously false because it is strongly dependent on the biases and extension of sampling (STEVENS, 1980). Even when these conditions of unbiased and sufficient sampling are met, a modal karyotype (in a wide sense) for a group, does not necessarily mean the most widespread karyotype among the supraspecific taxa of the group.

If taxon X is constituted, for example, of five tribes, M, N, O, P and Q, and karyotype I appears in 60% of the whole sampled species but is restricted to the tribe M, while the karyotype II appears in only 30% of the whole but in each of the five tribes, karyotype I is the modal and karyotype II is the most widespread in the higher taxa of X. This difference between modal and most widespread, together with the inclusion of a right outgroup and analysis of parsimony, is crucial to understanding any presumed polarity in evolutionary cytogenetics.

In an overview on the applications of molecular systematics HILLIS & MORITZ (1990) claim that cytogenetics is an appropriate and effective method to infer phylogenies among taxa whose divergences range from five to 50 million years (mya), but it is marginally appropriate or appropriate only under limited circumstances for lower time frames of divergence (0 to 5 mya) or higher (50 to 500 mya).

Therefore, only closely related species diverged within the past five mya and those that diverged since more than 50 mya, cannot be generally subjected to a cytogenetic analysis for estimating their phylogenies. Furthermore, SANTIAGO-BLAY (1994) reports that the fossil record for eight extant subfamilies of the Leaf Beetles goes back to Mesozoic (245 to 66.4 mya), and most of them first came in the Jurassic period (195 to 135 mya). Assuming these times of origin for a good number of Chrysomelidae subfamilies either in Jurassic or in Cretaceous periods, they fall apart from the range of time where cytogenetics is appropriate to deduce phylogenies. Four subfamilies or tribes of Leaf Beetles share a modal chromosome number of 2n = 16, Criocerinae, Pachybrachini (Cryptocephalinae), Eumolpinae and Hispinae (s. str.), although in agreement with most phylogenetic proposals there are no grounds to assume any direct phylogenetic relatedness among them, except maybe for Criocerinae and Hispinae (MANN & CROWSON, 1981; REID, 1995). Very likely, their common modal number should be explained as a simple convergence, at least as far as striking substantial evidence of allied phylogenetic interrelationships among them is true.

There is considerable controversy regarding ancestral/primitive state as either commonality or ancestral, as most widespread. With regard to the subfamilies of Chrysomelidae, and assuming the necessary premise of an adequate and representative sampling of karyotypes in each subfamily, the most widespread karyotype and meioformula, not the modal one by itself, can presumably be taken as the ancestral karyotype if it is the most parsimonious. This is also the opinion of MADDISON (1985) in his large study on the cytogenetics of *Bembidion* ground Beetles (Carabidae).

Among the different chromosomally examined subfamilies of Leaf Beetles, the amount of cytogenetic data is not sufficient to ascertain the most widespread karyotype in Eumolpinae since only four of the fourteen tribes (SEENO & WILCOX, 1982) have been surveyed. In Cryptocephalinae two of the five tribes (SEENO & WILCOX, 1982) are known, but each for only one genus. Their show very different modal numbers, *Cryptocephalus* (Cryptocephalini) 2n = 30, and *Pachybrachis* (Pachybrachini) 2n = 16 which precludes any phylogenetic assessment. This may be due to either the poor tribal screening or to the striking heterogeneity between the two previous genera. Three other subfamilies, Galerucinae, Alticinae and Cassidinae, are particularly diverse in higher taxonomic groups. Moreover, in Alticinae, the current tribes are so misleading (SEENO & WILCOX, 1982) that they also prevent any reliable attempt to determine their presumed ancestral karyotypes. Nevertheless, a valuable approach to the cytotaxonomy, but not the whole phylogeny of Alticinae, was published by VIRKKI (1988).

On the contrary, two additional subfamilies, Donaciinae and Criocerinae, with fewer species and higher taxa, have been sufficiently surveyed (PETITPIERRE et al., 1988, in prep.) to suggest their presumed most widespread karyotypes, 2n = 30 and 2n = 16, respectively. Finally, the subfamily Chrysomelinae, the best relatively well-known higher taxon of Leaf Beetles will be discussed in greater depth on continuation.

The phylogenetic significance of karyotype in Chrysomelinae

Of the roughly 3,000 described species (Daccordi, 1996), the meioformulas and/or karyotypes of Chrysomelinae have been determined for 203 species (Petitpierre et al. 1988; Petitpierre & Juan, 1994; Petitpierre, in prep.), which corresponds to 6.8% of the total group. Although the genus screening is not very large, in 30 of the 132 present genera (23%) (Daccordi, 1994, 1996) most current tribes and subtribes have been checked in a rather good sampling. On the other hand, a general agreement has been reached about the monophyly of Chrysomelinae (Reid, 1995; Daccordi, 1996), a favourable condition to perform their phylogenetic analysis.

Seeno & Wilcox (1982) followed the division of the subfamily in two tribes, Timarchini and Chrysomelini. The latter was further split into twelve subtribes, but the present taxonomic views based on reconsideration of adult characters and, pricipally, on a much better knowledge of the larval and pupal morphology, have reduced their number to only five subtribes: Timarchina (Timarchini), Entomoscelina, Paropsina, Chrysolinina and Chrysomelina (Daccordi, 1994, 1996). However, Steinhausen (1996) goes even further in joining Entomoscelina with Paropsina (=Gonioctenina), based on their larval and pupal characteristics, which does not seem substantiated until a much larger screening of immature stages in genera of Entomoscelina becomes available.

The diversity of haploid chromosome numbers for these five subtribes of Chrysomelinae is given in table 1. The modal number for the subfamily is n = 12 chromosomes, a value found in 84 (41.3%) of the total 203 checked species. This is the most frequent number in Chrysolinina (51.7% of species) and almost the only number displayed in Paropsina (96% of species), but it is very scarcely represented in Timarchina (3% of species), or in Chrysomelina (8% of species), and has not been observed to date in Entomoscelina. The Timarchina have n=10 as modal value (57% of taxa), the Entomoscelina have a mode at n=13 (83% of species) although the number of sampled species is still scanty, while that of Chrysomelina is n = 17 (48% of species). The modal value for the whole Chrysomelinae, n = 12, cannot be assumed as the ancestral value because it appears in only one species of Timarchini, the most primitive taxon of the subfamily in adult and larval morphology, life history and behaviour, and does not occur in Entomoscelina.

On the contrary, Chrysomelina, the most evolved subtribe in larval morphology, life history and behaviour (Paterson, 1931; Kimoto, 1962; Takizawa, 1976), has the highest modal number, n = 17, among the five subtribes of Chrysomelinae, which would probably imply the most advanced phylogenetic position. In summary, n = 10 is the most widespread chromosome number in Timarchini, and in the Cerambycidae, the right outgroup of Leaf Beetles, and due also to the correspondence with the remarkable primitiveness of the *Timarcha*, it can reasonably be taken as the ancestral value for the whole tribe Timarchini. However, as this number of n=10 has further been found in two species of Chrysolinina, one *Cosmogramma* from South America and one *Oreina* from Europe, it might also be the ancestral number for Chrysolinina too. Nevertheless in accordance with the parsimony rules and assuming n = 12 as the ancestral number for Chrysolinina, it is much easier to explain the origin of the previous n = 10 chromosome species by one centric fusion in each, rather than the reverse, that is, the derivation of 59 species with n = 12 chromosomes from those by a clearly higher figure of centric fissions. The same reasoning could be applied in trying to ascertain the presumed ancestral number for Chrysomelina, with n = 17 chromosomes the most widespread, and in agreement with their derived morphological and behavioural features, it can be rightly taken as the ancestral karyotypic value for the subtribe.

Phenotypic correlates of karyotypes in Chrysomelinae

The larval morphology of Chrysomelinae offers excellent characters to set up groups of species more clearly differentiated than their corresponding adult stages.

Table 1. Distribution of haploid chromosome numbers among higher taxa of Chrysomelinae Leaf Beetles: T. Timarchini; Chrl. Chrysolinina (including six polyploid parthenotes of *Calligrapha*); P. Paropsina; E. Entomoscelina; Chrm. Chrysomelina; gen. Genera; sp. Species

Distribución de los números de cromosomas haploides en los grandes taxones de Chrysomelinae. (Para abreviaturas ver arriba.)

	Examined		Haploid numbers																		
	gen.	sp	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
T	1	35	-	-	-	1	20	2	1	5	4	1	-	-	-	-	-	-	1	-	-
Chrl	12	114	1	-	-	-	2	3	59	1	6	1	3	5	15	2	10	3	-	2	1
P	6	23	-	-	-	-	-	-	22	-	-	-	-	-	-	-	1	-	-	-	-
E	3	6	-	-	-	-	-	-	-	5	1	-	-	-	-	-	-	-	-	-	-
Chrm	8	25	-	-	-	-	-	-	2	-	2	4	5	12	-	-	-	-	-	-	-
Total	30	203	1	-	-	1	22	5	84	11	13	6	8	17	15	2	11	3	1	2	1

Three large groups of larvae are generally recognized by several authors (PATERSON, 1931; HENNIG, 1938; KIMOTO, 1962; TAKIZAWA, 1976; COX, 1982): 1. Non-tuberculate larvae (Timarchini); 2. Tuberculate but 'non-glanduliferous' larvae, having only one pair of defensive glands (Chrysolinina, Entomoscelina and Paropsina, including the former Gonioctenina); 3. Tuberculate and glanduliferous larvae, with nine pairs of glands (Chrysomelina, including the former Phyllodectina). These three types of larvae are rather well correlated with the recent results of chemotaxonomy which have been discussed and summarized by PASTEELS & ROWELL-RAHIER (1989) and PASTEELS (1993). Thus, in the subtribe Chrysomelina, all the studied genera and species, except for one which is misclassified, secrete nitropropanoic acid and isoxazolinone glucosides, while Chrysolinina-Doryphorina-Gonioctenina produce cardenolides, ethanolamine or amino acid derivatives, with the exception of the *Chrysolina* subgenus *Hypericia*, which is characterized by secreting polyoxygenated steroid glycosides as well as ethanolamine. Finally, the ancestral tribe Timarchini is strikingly separated from the previous taxa by its probable secretion of anthraquinones as defensive substances (PETITPIERRE, 1995). The three main types of larvae and their defensive allomones correspond with the three basic modal karyotypes of Timarchini (n = 10), Chrysolinina and Gonioctenina (n = 12), and Chrysomelina (n = 17). Another interesting insight of cytotaxonomic value comes from genome size, since the species of Chrysomelina have significantly smaller genomes than those of the other subtribes, very likely an apomorphic condition of these most advanced chrysomelines (PETITPIERRE & JUAN, 1994).

All these features of phylogenetic value have also been validated by the analyses of nucleotide sequences of mitochondrial DNA (HSIAO, 1994a, 1994b). This has allowed some phylogenetic trees to be built up, where the genera of Chrysomelina clearly branched off from those of Chrysolinina-Doryphorina. Nevertheless, in the second and more extensive paper (HSIAO, 1994b), only one species of Timarchini was included as an outgroup, and the few checked Paropsina (3 spp.) and Entomoscelina (1 sp.), were not clearly clustered. A much larger screening of these three higher taxa is therefore necessary for a more conclusive picture of their genetic relatedness.

Some clues to explain the karyological diversity of Leaf Beetles

All chromosomal shifts which may be detected either inter- or intraspecifically depend on mutation as a primary factor. This means that any new mutant is heterozygous for such a chromosomal change, and assuming that this mutation does not negatively affect the survival of the mutant individual, it can only be kept and spread if it becomes fixed. Hence it becomes a homozygous condition. Several factors can account for the fixation of those chromosomal shifts without deleterious effects causing their elimination by natural selection. BENGTSSON (1980) has detailed the five most important models leading to fixation of chromosome mutations: 1. Segregation distortion (or meiotic drive in a more frequently used name); 2. Selective advantage; 3. Recombination modification; 4. Inbreeding and homozygote advantage; 5. Random genetic drift. Among these five models the last two have attracted more interest and attention than the others. CHESSER & BAKER (1986) have developed computer simulation models to determine the conditions for the stochastic fixation of chromosomal mutations in small isolated demes. These models predict fixation under the conditions of: a. A small number of initial founders (5 or 10); b. A relatively small reduction in fecundity due to meiotic problems; and c. A high number of offspring per mating. Moreover, LANDE (1985) estimated the capacity of fixation of a new chromosomal change through colonization of further demes by the mutant individuals.

These conditions can be applied to the Leaf Beetles as an approach to understanding their chromosomal evolution. Effective population size is a crucial factor in order to explain the fixation of chromosomal changes due to inbreeding and genetic drift. Although direct measures of population sizes in Leaf Beetles are lacking, some indirect features may provide valuable information in this regard. The flying capacity versus flightlessness is a character with a presumed immediate effect on the dispersal potentiality of a species. In a broad sense, the local population or deme size is probably enlarged in the former and reduced in the latter. Furthermore, the oligophagous or monophagous versus polyphagous feeding selection is very likely related to the deme size, being much larger in the latter. One very interesting study on insect numbers has demonstrated increased opportunities for speciation in the specialized phytophagous insects respect to the non-phytophagous insect taxa (MITTER et al., 1988). In our particular frame of Chrysomelinae Leaf Beetles, we chose twelve genera with a minimum number of four chromosomally examined species to test the previous hypotheses. A significant correlation was found between the rate of chromosomal evolution, given as standard deviation of diploid numbers, and the species richness per genus ($r = 0.53$). A much higher correlation was obtained between the rate of chromosomal evolution per genus and the number of host plant families selected by these congeneric species ($r = 0.84$), but there were no clear-cut differences in the chromosomal evolution between flying and flightless species genera (PETITPIERRE et al., 1993). Therefore, the oligophagous or monophagous feeding preferences increase the chance for chromosomal evolution and speciation, because the conditions suggested by CHESSER & BAKER (1986) are probably fulfilled, although the interrelationship between chromosomal evolution and speciation is a matter of debate, and indeed not necessarily univocal. Furthermore, if examples of meiotic drive in Leaf Beetles are reported in the next years, we will have more valuable information to understand their karyological evolution and the processes leading to fixation of new chromosomal variants.

Resumen

El valor de la citogenética para la taxonomía y la evolución de los crisomélidos (Coleoptera, Chrysomelidae)

Se discuten en detalle las ventajas e inconvenientes de la citogenética para la taxonomía y estudio de la evolución de los crisomélidos. La cariología puede suministrar medios para distinguir especies gemelas crípticas como se demuestra en *Chrysolina*

aurichalcea y *Cassida viridis*. El valor filogenético de los cariotipos solo puede invocarse en base a tres reglas generales: un amplio muestreo para conocer el cariotipo o meiofórmula más extendida, el criterio de parsimonia, y la evolución paralela en otros caracteres. Desde luego, un cariotipo modal no puede ser considerado a priori como el más primitivo, por tanto, por simples razones de muestreo, sólo puede reconocerse el cariotipo ancestral en unas pocas subfamilias. Entre ellas los Donaciinae, con 2n = 30 (Xy_p) y los Criocerinae, con 2n = 16 (Xy_p), parecen estar razonablemente establecidos, pero no los Cryptocephalinae, Eumolpinae, Galerucinae, Alticinae, Hispinae y Cassidinae, porque aunque se han estudiado bastantes especies de todas estas subfamilias, en todas ellas quedan todavía muchas tribus o grupos de géneros, como sucede en los Alticinae, sobre las cuales no hay datos cromosómicos. La subfamilia Chrysomelinae, con casi un 7% de sus 3000 especies caracterizadas cromosomicamente, permite un análisis más profundo, para discutir las posibles relaciones de sus tribus y subtribus basadas en los datos citogenéticos y las claras correspondencias observadas con otros caracteres de valor filogenético. También se tratan los probables efectos del tamaño de las poblaciones locales y de la especialización trófica sobre plantas hospedadoras, en la evolución cromosómica de los géneros de Chrysomelinae.

References

BENGTSSON, B. O., 1980. Rates of karyotype evolution in placental mammals. *Hereditas*, 92: 37-47.

CHESSER, R. K. & BAKER, R. J., 1986. On factors affecting the fixation of chromosomal rearrangements and neutral genes: computer simulations. *Evolution*, 40: 625-632.

COX, M. L., 1982. Larvae of the British genera of chrysomeline beetles (Coleoptera, Chrysomelidae). *Syst. Ent.*, 7: 297-310.

CROWSON, R. A., 1981. *Biology of the Coleoptera*. Academic Press.

- 1994. Preface. A long perspective on chrysomelid evolution. In: *Novel aspects of the biology of Chrysomelidae:* XIX-XXIII (P. H. Jolivet, M. L. Cox & E. Petitpierre, Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

DACCORDI, M., 1994. Notes for phylogenetic study of Chrysomelinae, with descriptions on new taxa and a list of all the known genera (Coleoptera: Chrysomelidae, Chrysomelinae). In: *Proceedings of the Third International Symposium on the Chrysomelidae, Beijing 1992*: 60-84 (D. G. Furth, Ed.). Backhuys Publ., Leiden (Netherlands).

- 1996. Notes on the distribution of the Chrysomelinae and their possible origin. In: *Chrysomelidae Biology, vol. 1*: 399-412 (P. H. A. Jolivet & M. L. Cox, Eds.), SPB Academic Publ., Amsterdam.

FUJIYAMA, S., 1989. Species problems in *Chrysolina aurichalcea* (Mannerheim) with special reference to chromosome numbers. *Entomography*, 6: 443-452.

FUJIYAMA, S., SASAWAKI, A. & NAGAO, M. 1991. Geographic distribution of the two types of chromosome number and frequency of the colour forms in *Chrysolina aurichalcea* complex in Nagano Prefecture, central Honshu, Japan, with special reference to natural environment. *Ann. Environ. Sci., Shinshu Univ.*, 13: 21-31.

FUJIYAMA, S. & TAKANASHI, H., 1994. Geographic distribution of two types of chromosome number in *Chrysolina aurichalcea* complex and their phenotypic frequencies of the two color forms in Kanto district, Japan. *Ann. Environ. Sci., Shinshu Univ.*, 16: 53-58.

HENNIG, W., 1938. Ubersicht über die wichtgsten deutschen chrysomelinen. *Arbeiten Physiologische Angewandte Entomologische*, 5: 85-136.

HILLIS, D. M. & MORITZ, C., 1990. An overview of applications of molecular systematics. In: *Molecular Systematics*: 502-515 (D. M. Hillis & C. Moritz, Eds.). Sinauer Associates, Sunderland.

HSIAO, T. H., 1994a. Molecular techniques for studying systematics and phylogeny of Chrysomelidae. In: *Novel aspects of the biology of Chrysomelidae:* 237-248 (P. H. Jolivet, M. L. Cox & E. Petitpierre, Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

- 1994b. Molecular phylogeny of chryso-

melid beetles inferred from mitochondrial DNA sequence data (Coleoptera: Chrysomelidae). In: *Proceedings of the Third International Symposium on the Chrysomelidae, Beijing 1992*: 9-17 (D. G. Furth, Ed.). Backhuys Publ., Leiden (Netherlands).

HSIAO, T. H. & HSIAO, C., 1983. Chromosomal analysis of *Leptinotarsa* and *Labidomera* species (Coleoptera: Chrysomelidae). *Genetica*, 60: 139-150.

JOLIVET, P. H. A. & COX, M. L. (Eds.), 1996. *Chrysomelidae Biology*, 3 vols. SPB Acad. Publ., Amsterdam.

JOLIVET, P. H., COX, M. L. & PETITPIERRE, E. (Eds.), 1994. *Novel aspects of the biology of Chrysomelidae*. Kluwer Acad. Publ., Dordrecht (Netherlands).

JOLIVET, P., PETITPIERRE, E. & HSIAO, T. H. (Eds.), 1988. *Biology of Chrysomelidae*. Kluwer Acad. Publ., Dordrecht (Netherlands).

KIMOTO, S., 1962. A phylogenetic consideration of Chrysomelinae based on immature stages of Japanese species. *J. Fac. Agric. Kyushu Univ.*, 12: 67-114.

LANDE, R., 1985. The fixation of chromosomal rearrangements in a subdivided population with local extinction and colonization. *Heredity*, 54: 323-332.

LAWRENCE, J. F., 1982. Coleoptera. In: *Synopsis and classification of living organisms, vol. 2*: 482-553 (J. A. Parker, Ed.). McGraw-Hill, New York.

LEE, J. E., 1993. Phylogenetic studies on the larvae of the Chrysomelidae (Coleoptera) from Japan. *Jpn. J. Ent.*, 61: 409-424.

MADDISON, D. R., 1985. Chromosomal diversity and evolution in the ground beetle genus *Bembidion* and related taxa (Coleoptera: Carabidae: Trechinae). *Genetica*, 66: 93-114.

MANN, J. S. & CROWSON, R. A., 1981. The systematic positions of *Orsodacne* Latr. and *Syneta* Lac. (Coleoptera Chrysomelidae), in relation to characters of larvae, internal anatomy and tarsal vestiture. *J. Nat. Hist.*, 15: 727-749.

MITTER, C., FARRELL, B. & WEIGMANN, B., 1988. The phylogenetic study of adaptive zones: has phytophagy promoted insect diversification? *Amer. Nat.*, 132: 107-128.

PASTEELS, J. M., 1993. The value of defensive compounds as taxonomic characters in the classification of leaf beetles. *Biochem. Syst. Ecol.*, 21: 135-142.

PASTEELS, J. M. & ROWELL-RAHIER, M., 1989. Defensive glands and secretions as taxonomic tools in the Chrysomelidae. *Entomography*, 6: 423-432.

PATERSON, N. F., 1931. The bionomics and comparative morphology of the early stages of certain Chrysomelidae (Col. Phyt.). *Proc. Zool. Soc. Lond.*, 4: 879-940.

PETITPIERRE, E., 1981. New data on the cytology of *Chrysolina* (Mots.) and *Oreina* Chevr. (Coleoptera, Chrysomelidae). *Genetica*, 54: 265-272.

- 1983. Karyometric differences among nine species of the genus *Chrysolina* Mots. (Coleoptera, Chrysomelidae). *Can. J. Genet.Cytol.*, 25: 33-39.
- 1995. Presence of anthraquinones in the haemolymph of *Timarcha* (Coleoptera; Chrysomelidae; Chrysomelinae). *Chrysomela Newsletter*, 30: 4.

PETITPIERRE, E. & JUAN, C., 1994. Genome size, chromosomes and egg-chorion ultrastructure in the evolution of Chrysomelinae. In: *Novel aspects of the biology of Chrysomelidae:* 213-225 (P. H. Jolivet, M. L. Cox & E. Petitpierre, Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

PETITPIERRE, E., JUAN, C., FUJIYAMA, S. & CARRERAS, I., 1991. Las especies gemelas del complejo *Chrysolina aurichalcea* (Coleoptera, Chrysomelidae): divergencia genética y evolución. In: *XXVI Jorn. Luso-Espanholas de Genética (Coimbra)*: 96. Univ. de Coimbra, Coimbra.

PETITPIERRE, E., SEGARRA, C. & JUAN, C., 1993. Genome size and chromosomal evolution in leaf beetles (Coleoptera, Chrysomelidae). *Hereditas*, 119: 1-6.

PETITPIERRE, E., SEGARRA, C., YADAV, J. S. & VIRKKI, N., 1988. Chromosome numbers and meioformulae of Chrysomelidae. In: *Biology of Chrysomelidae*: 161-186 (P. Jolivet, E. Petitpierre & T. H. Hsiao Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

REID, C. A. M., 1995. A cladistic analysis of subfamilial relationships in the Chrysomelidae *sensu lato* (Chrysomeloidea). In: *Biology, Phylogeny and Classification of Coleoptera: Papers Celebrating the 80th Birthday of Roy A. Crowson, vol. 2*: 559-631(J. Pakaluk & S.A. Slipinski, Eds.). Muzeum Instytut Zoologii PAN, Warszawa, Poland.

SANTIAGO-BLAY, J. A., 1994. Palentology of leaf beetles. In: *Novel aspects of the biology of Chrysomelidae*: 1-68 (P. H. Jolivet, M. L. Cox & E. Petitpierre, Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

SEENO, T. N. & WILCOX, J. A., 1982. Leaf Beetle genera (Coleoptera: Chrysomelidae). *Entomography*, 1: 1-221.

SMITH, S. G. & VIRKKI, N., 1978. *Coleoptera*. In: *Animal Cytogenetics Vol. 3. Insecta 5* (B. John, Ed.). Gebruder Borntraeger, Berlin-Stuttgart.

STEINHAUSEN, W., 1996. Vergleichende morphologie der puppen der blattkäferunterfamilie Chrysomelinae (Col., Chrysomelidae). *Ent. Nach. Berich.*, 40: 89-98.

STEVENS, P. F., 1980. Evolutionary polarity and character states. *Ann. Rev. Ecol. Syst.*, 11: 333-358.

TAKIZAWA, H., 1976. Larvae of the genus *Gonioctena* Chevrolat (Coleoptera: Chrysomelidae): Descriptions of Japanese species and the implications of larval characters for the phylogeny. *Kontyû, Tokyo*, 44: 444-468.

VIRKKI, N., 1988. Cytotaxonomy of Alticinae. In: *Biology of Chrysomelidae:* 187-203 (P. Jolivet, E. Petitpierre & T. H. Hsiao, Eds.). Kluwer Acad. Publ., Dordrecht (Netherlands).

WHITE, M. J. D., 1978. *Modes of Speciation*. W. H. Freeman, San Francisco.

YADAV, J. S. & PILLAI, R. K., 1975. Karyological studies on four species of Cassidinae (Coleoptera: Chrysomelidae). *Genen Phaenen*, 18: 55-63.